CHLOROSIS AND LACK OF VIGOUR IN SEEDLINGS OF RENANTHEROUS SPECIES OF *EUCALYPTUS* CAUSED BY LACK OF MYCORRHIZA.

By L. D. Pryor.

(Plate ix; one Text-figure.)

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Synopsis.

Poor growth and chlorosis in seedlings of some Renantherous species of *Eucalyptus* are corrected by the addition of *Scleroderma flavidum* spores, which leads to the formation of an ectotrophic mycorrhiza. Records of introduction in the northern hemisphere suggest that lack of suitable mycorrhiza may be responsible for the poor growth of the Renantherae as a whole. Obligate mycorrhiza in *Eucalyptus* seems to be limited to the systematic group Renantherae. It is suggested that a retrial, with the addition of fungal spores, of the important Renantherous species in areas where they have failed but might be expected to grow because of soil and climate, would be worth while.

It was noticed in 1953, when attempting to raise various species of *Eucalyptus* in the nursery at Eski Kellek, in Iraq, that some valuable species, all belonging to the systematic group, the Renantherae, grew weakly and in many cases became chlorotic and often died in the nursery soon after the second or third pair of leaves had been produced. This was found to apply to *Eucalyptus dives*, *E. pauciflora* and *E. Robertsonii*. The growth of *E. fastigata*, also in the same group, was a little better than that of the other three species but still was far from thrifty.

It was thought at the time, when looking for an explanation, that the highly calcareous soils characteristic of much of Iraq may have contributed to the trouble. The view that chlorosis and poor growth or even death might be caused, perhaps indirectly, by excess lime, had some support in the evidence from *E. camaldulensis*. This is the most commonly planted Eucalypt through the Mediterranean region and the Middle East. It thrives in many localities, but it is noticeable while tolerating some degree of lime that on highly calcareous soils in Iraq, Israel, Jordan and Morocco it becomes at times very chlorotic and fails. It has been suggested recently by work in Israel (Karschon, 1953), that chlorosis in this species can be corrected by suitable iron injection and that the effect is caused by lime-induced iron deficiency.

Some of the species which were raised successfully to the eighth leaf-pair stage in Iraq at the same time, in the nursery under the same conditions, included *E. microcarpa*, *E. sideroxylon*, *E. bicostata*, *E. viminalis* and *E. gomphocephala*, as well as *E. camaldulensis*. It could then be implied, if lime were the cause of the trouble, that the species concerned which failed must be distinctly more susceptible than *E. camaldulensis*.

Since nothing was known of the response of *Eucalyptus* to various major fertilizers, trials were made in Canberra with *E. macrorrhyncha* (Renantherae) and *E. bicostata* (Macrantherae) in pots of unsterilized washed sand to which ammonium sulphate, potash, superphosphate and lime were added singly at two ratings, and potash, superphosphate and ammonium sulphate together. A dressing in the form of ground calcium carbonate was used for the lime treatment. The response of both species to ammonium sulphate was marked, even when provided alone. The effect of lime was firstly to reduce germination to some degree, and of the plants which did become established the growth was comparable with the control, although there was a tendency for a purplish colour to develop at the margins and tips of the leaves. There was, however, no sign of chlorosis in the young plants and growth was reasonably healthy and not very different from that in which potash and superphosphate were supplied each alone. The results of these simple experiments, therefore, suggested that the chlorosis developed in Iraq was not induced by excess lime.

Trials were then made in Canberra growing *Eucalyptus* under bell jars in electrically heat-sterilized soil. A chlorotic condition similar to that found in Iraq developed in the Renantherous species, particularly in *E. pauciflora*, *E. macrorrhyncha*, *E. dives* and *E. fastigata*, while *E. bicostata* (Macrantherae) and *E. melliodora* (Terminales) did not show these symptoms. A further test was made by transplanting from sterilized soil eight highly chlorotic seedlings of *E. pauciflora* (in the second leaf-pair stage), four to non-sterilized soil and four to sterilized soil. In the space of three weeks the plants transferred to non-sterilized soil developed green leaves and regained vigour, while those transferred to sterilized soil lived for about five weeks and then died. On the roots of the plants in sterilized soil there was no trace of fungal hyphae, whereas in the unsterilized soil there was a thin, irregular investiture of hyphae.

Experiments were therefore extended to include non-sterilized soil. *E. pauciflora* and *E. macrorrhyncha* (Renantherae) were grown in comparison with *E. melliodora* (Terminales), *E. bicostata* (Macrantherae) and tomato. The growth of the latter three species (including tomato) was very substantially better in the sterilized than in the non-sterilized soil. On the other hand, the growth of *E. pauciflora* and *E. macrorrhyncha* was healthy and somewhat faster in the non-sterilized soil than in the sterilized soil, where it was chlorotic and weak. It is also noticeable with heat-sterilized soil in normal nursery practice that species of the Renantherous group are particularly prone to chlorosis and this frequently leads to death. When the species are grown in open-ended tubes in the nursery, however, they often grow well enough to root through to the soil below the tubes and then make a substantial recovery. It was concluded that heat sterilization of soil (taken from beneath a *Eucalyptus* stand) makes available nutrients of which only species of the Macrantherae and Terminales groups can immediately make use. It also seemed from the tests that the chlorotic condition which develops was probably due to lack of mycorrhiza due to the destruction by sterilization of the essential fungal symbiont.

Trials by Adding Fungal Spores.

Reports of mycorrhizal association in *Eucalyptus* are confined to morphological or anatomical descriptions, and no demonstration has been made hitherto to suggest that any such association is either obligatory or even beneficial. Samuel (1926) recorded an ectotrophic mycorrhiza on *E. rubida*. Material from *E. gigantea* from a natural stand examined by N. H. White (1954) appeared very characteristically mycorrhizal, showing the short, much-branched coralloid type of root formation with a distinct ectotrophic mycorrhiza exhibiting the characteristic "Hartig net". Smith and Pope (1934) record ectendotrophic mycorrhiza on species of *Eucalyptus* (unnamed) in South Africa, formed by a South African species of *Polysaccum* (syn. *Psilolithus*). The common occurrence of a range of Gasteromycetes in *Eucalyptus* forest has suggested mycorrhiza forming fungal species may be found in this group.

It was noticed in trial *Eucalyptus* plantations that there was a high degree of association between the fruiting bodies of *Scleroderma flavidum* and planted trees, and particularly with the species *E. pauciflora* and *E. Robertsonii* of the Renantherae. This was best seen after certain weather conditions following clean cultivation when the Gasteromycete disclosed its presence by cracking the surface of the soil. Fruiting bodies of this fungus were collected and an experiment was carried out in which spores were added to heat-sterilized soil in pots. It was realized, of course, that this method falls short of a pure culture technique, but it was thought that if the usual conditions of mycorrhiza formation with a Gasteromycete applied, an indication of the significance of the *Scleroderma* might be obtained. The pots with soil were heat-sterilized in an electric oven at about 400°F. for about five hours. The trial species were *E. bicostata* (Macrantherae), and *E. macrorrhyncha*, *E. pauciflora* and *E. dives* (Renantherae). Five pots of each were used. In the treated pots a liberal application of the spores of *Scleroderma* was made, sowing being carried out after an additional half an inch of sterilized soil had been added to cover the spores. Seed to produce about 100 seedlings was sown in each pot. The sowing was carried out in late winter and the pots placed in a heated frame to hasten growth. The conditions in the frame were more favourable for growth than in the earlier experiments under bell jars.

Differences appeared between the treated and untreated pots after about four months and at eight months this was marked. The untreated showed general discoloration and unthrifty growth in the Renantherous species, whereas the treated were much better in colour and growth (Pl. ix, fig. 1). There was no effect of inoculation on *E. bicostata* (see Table 1). Examination of the roots showed that in the pots to which the Scleroderma spores had been added distinct mycorrhizal clusters were visible both on the outside of the ball and within the ball itself. The clusters have the distinct morphological appearance of ectotrophic mycorrhiza with a short coralloid branching habit and a prominent whitish hyphal sheath (Pl. ix, figs. 2 and 3). There was no sign of this mycelium or mycorrhiza in the untreated pots. Microscopic examination of a mycorrhiza from one of the *E. macrorrhyncha* pots showed a thick hyphal mantle with distinct Hartig net penetrating to a depth of two or three cortical cells (Text-fig. 1). It is concluded, therefore, that *Scleroderma flavidum* can act as a mycorrhizal-forming fungus capable of promoting thrifty growth in the three Renantherous species of *Eucalyptus* tested, whereas, in the absence of this fungus, growth of these species in heat-sterilized soil is poor and unthrifty.

TABLE 1.

		A. Heat Sterilized Soil+Scleroderma Spores.			B. Heat Sterilized Soil.		
		Height Growth.* (6 mos.).	Colour.	Mycorrhiza.	Height Growth (6 mos.).	Colour.	Mycorrhiza.
		In.			In.		
Renantherae.	*E. dives*	5·3	Good.	Present.	3·2	Yellowish.	Nil.
	E. pauciflora	6·2	Good.	Present.	3·3	Yellowish.	Nil.
	E. macrorrhyncha	11·3	Good.	Present.	7·8	Yellowish.	Nil.
Macrantherae.	*E. bicostata*	9·5	Good.	Nil.	8·8	Good.	Nil.

* Average maximum growth. There were many seedlings per pot.

DISCUSSION.

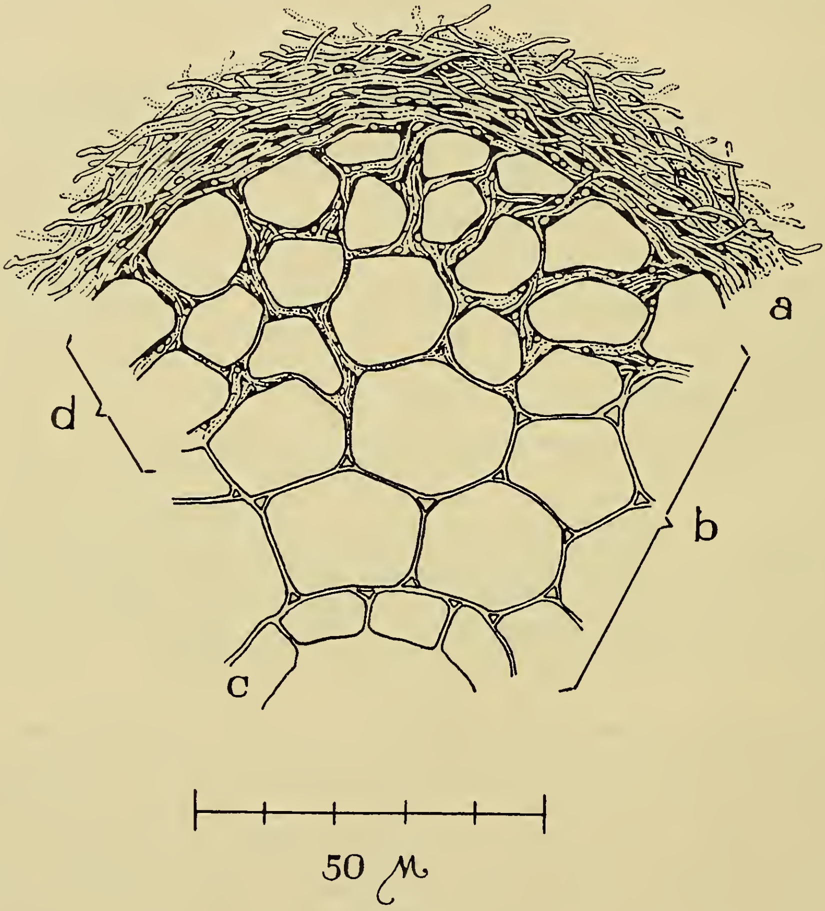
The precise role of mycorrhiza in *Eucalyptus* is likely to be as complex as in other tree species and doubtless is bound up with nutrition of the plant. It appears that with the Renantherous group of *Eucalyptus* species the presence of mycorrhiza is essential for healthy growth and that chlorosis in the Renantherae is generally due to lack of a suitable fungal symbiont. There is no reason to suppose that many other species or genera of fungi, besides *Scleroderma*, may not be similarly associated. According to Burges (1936), Peyronel lists 13 species of fungi which form mycorrhiza on *Fagus sylvatica*, and Melin (1948) mentions that about 30 species of fungus have so far been proved to be mycorrhiza forming with *Pinus sylvestris*.

Scleroderma as a genus is considered a mycorrhizal fungus of a good many genera in the northern hemisphere, particularly *Larix*, *Castanea*, *Fagus* and *Quercus* (Rayner, 1927). The one species used in this test has been determined as *Scleroderma flavidum*, which is found in Australia, New Zealand, Africa and America, the type having been described from America. The distribution in both northern and southern hemispheres is in conflict with the idea that suitable mycorrhizal fungi are lacking in the north, but it is likely that even if they are the one species (which is uncertain because of the difficulties in the taxonomy of the Gasteromycetes), the Australian form is physiologically distinct.

The most notable exception to the failure of the Renantherous species outside Australia is with *E. fastigata*, *E. regnans*, *E. obliqua* and *E. piperita* in New Zealand

and a few other species in South Africa. In view of the fact that on the one hand New Zealand is known to share with Australia one species of *Scleroderma* which is confined to the two countries, and the fact that there are strong vegetational links otherwise with South Africa and New Zealand which may well extend to the fungi, the explanation could be that adequate mycorrhizal symbionts are already available in those two countries, and Smith and Pope's (ibid.) observations lend support to this view.

In the northern hemisphere the position is different and a review of the record of introductions of *Eucalyptus* as an exotic shows that poor growth or failure is experienced with species of the Renantherae in many plantation projects outside Australia. Although it is known that many species of this group have been tried overseas, very few have made successful growth. An exception is *E. coccifera* and perhaps *E. gigantea* on the west coast of Scotland (Martin, 1948). Of course in some cases failure is simply due to the fact that the climate and soil are unsuitable, but this is by no means always so. Results from trials by M. Menager (1953), who has planted Eucalypts in Les Landes, France, are interesting in this connection. He notes as a result of his trials over four or five years that the best species are *E. Gunnii*, *E. Macarthuri*, *E. subcrenulata*, *E. rubida*, *E. Dalrympleana*, *E. viminalis*,



Text-figure 1.

Cross section of a mycorrhiza of *E. macrorrhyncha* and *Scleroderma flavidum* showing (*a*) hyphal mantle, (*b*) cortex, (*c*) endodermis, and (*d*) Hartig net.

E. stellulata, *E. parvifolia*, *E. bicostata* and *E. camaldulensis*, whereas perhaps the most valuable ones which have largely or entirely disappeared are *E. gigantea*, *E. obliqua*, *E. regnans*, *E. Andreana* (syn. *E. Lindleyana*), *E. Robertsonii* and *E. pauciflora.* It is striking that of these two groups the first belong to the Macrantherae with the one exception, *E. stellulata*, and the second entirely to the Renantherae.

In Cyprus there is a similar situation. Chapman (1953) records 21 species of the Renantherae and 19 species of the Macrantherae-Normales as having been introduced by seed between 1878 and 1934, as well as species from other groups, making a total of some 70 species. It is true that in the areas in which Eucalypts have been planted in Cyprus, the rainfall is rather low and species from groups other than the Renantherae and Macrantherae-Normales are likely to thrive better. Nevertheless, five species of the Macrantherae-Normales out of the 19 recorded as having been introduced are now found growing on the island, whereas not a single specimen of the group Renantherae has survived, although 11 of those introduced are considered likely to succeed from the general point of view of climate and soil available. This evidence also supports the view that failure is due to lack of mycorrhiza.

The Renantherae as a group on genetic grounds have the status of a sub-genus within *Eucalyptus.* They have many features which cut them off very distinctly from all other systematic groups within the genus and there is evidence of a strong barrier to interbreeding between them and other groups of the genus (Pryor, 1951). That the Renantherae have ectotrophic mycorrhiza obligatory for thrifty growth is yet another distinctive character of this group.

To understand the precise role of mycorrhiza throughout all the species, the fungi involved and the various aspects of the way in which each is related to the growth of the tree would require extensive investigation. The present study suggests that a retrial of all the Renantherous species of outstanding importance as timber producers is justified in areas where they have failed but where from general considerations of climate and soil they might otherwise have been expected to succeed. A simple way to start would be by the addition of spores of *Scleroderma flavidum* from Australia to potting soil overseas, but it must be anticipated that several other fungi will be necessary to suit all species.

The most important species of the Renantherae which come into this category are *E. Andreana*, *E. Andrewsii*, *E. Blaxlandi*, *E. coccifera*, *E. dives*, *E. fastigata*, *E. fraxinoides*, *E. gigantea*, *E. globoidea*, *E. laevopinea*, *E. macrorrhyncha*, *E. marginata*, *E. Muelleriana*, *E. obliqua*, *E. oreades*, *E. pauciflora*, *E. pilularis*, *E. radiata*, *E. regnans*. *E. Robertsonii*, *E. salicifolia*, *E. scabra*, *E. Sieberiana*, *E. stellulata*, *E. triantha*, and *E. urceolaris*.

A further point of interest follows from the recognition of ectotrophic mycorrhiza on *Eucalyptus.* This is in conflict with Asai's (1934) view that ectotrophism is a rather primitive state of symbiosis because he found it confined to supposedly primitive dicotyledonous orders such as Salicales and Fagales. The order Myrtiflorae, to which *Eucalyptus* belongs, is considerably advanced in the Engler and Prantl scheme. Even within the genus itself there is no reason to suppose that the Renantherae is a primitive group, and Garratt's (1950) idea that ectotrophic mycorrhiza results from highly evolved root-infecting fungi fits the case better than Harley's (1948) implication that it is a partial specialization preceding the development of full endotrophism. Obligate ectotrophism seems to be a highly specialized condition and therefore, in this respect at least, the Renantherae would be the most advanced group of the genus.

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References.

ASAI, T., 1934.—Ueber das Vorkommen und die Bedeutung der Wurzelpilze in den Landpflanzen. *Japan. Journ. Bot.*, 7: 107-150.

BURGES, A., 1936.—On the significance of mycorrhiza. *New Phytol.*, 35: 119.

CHAPMAN, E. F., 1953.—The Cyprus Eucalypts. Nicosia.
GARRATT, S. D., 1950.—*Bio. Rev. Camb. Phil. Soc.*, 25(2) : 220-254.
HARLEY, J. L., 1948.—Mycorrhiza and Soil Ecology. *Biol. Rev. Camb. Phil. Soc.*, 23(2) : 127-158.
KARSHON, M. R., 1953.—Recherches sur la chlorose de l'eucalyptus. *Israel.*
MARTIN, D., 1948.—Eucalyptus in the British Isles. *Australian Forestry*, 12 : 63-74.
MELIN, E., 1948.—Recent Advances in the Study of Tree Mycorrhiza. *Trans. Brit. Mycol. Soc.*, 30 : 92-99.
MENAGER, H., 1953.—Personal communication.
PRYOR, L. D., 1951.—PROC. LINN. SOC. N.S.W., 76 : 140.
RAYNER, M. C., 1927.—Mycorrhiza. *New Phytologist Reprint*, No. 15, London.
SAMUEL, G., 1926.—*Trans. & Proc. Roy. Soc. South Australia*, 50 : 245.
SMITH, N. J. G., and POPE, F. B., 1934.—*Trans. Brit. Mycol. Soc.*, 19 : 95.
WHITE, N. H., 1954.—Personal communication.

EXPLANATION OF PLATE IX.

1. *E. dives.* Left, with spores of *Scleroderma*; right, in sterilized soil without spores.
2. Mycorrhizae of *Scleroderma* and *E. macrorrhyncha* on the outside of the root ball.
3. Mycorrhizae of *Scleroderma* and *E. macrorrhyncha* on the outside of the root ball.